Analysis of Excessive Homozygous Regions in Rhodesian Ridgeback Dogs

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Summary

The Rhodesian Ridgeback is an African hunter breed characterized by its dorsal fur ridge. DNA from 24 Austrian Ridgeback dogs was genotyped using 173,662 single nucleotide polymorphism (SNP) markers to detect runs of homozygosity (ROH). The detected ROH regions were used to calculate individual genomic inbreeding coefficients and detect excessively homozygous regions of the genome, also known as ROH islands. These islands common in the population could be also interpreted as signatures of selection. Indeed, one of the regions harbored genes defining breed specific appearance. The ridge gene complex (FGF3, FGF4, FGF19, ORAOV1), the defining characteristic of the Rhodesian Ridgeback dogs, were detected in a ROH island on chromosome 18. Additional identified genes located within the ROH islands were HMGA2 affecting body size, MSRB3 coding for floppy ears and MSTN for muscling patterns. Numerous health related genes were identified in the homozygous regions, that are proven to play a role in dog diseases, along with other genes described only in other species.

Key words

dog, SNP, inbreeding, run of homozygosity, selection signatures

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Introduction

The Rhodesian Ridgeback is an FCI approved dog breed that received its name from a dorsal fur ridge what is branded by hair growth in the opposite direction. The breed originates from the region of Zimbabwe, formerly Rhodesia. It evolved from crosses from Hottentot dogs with numerous European breeds and was standardized in 1922. According to the definition they are muscular, active dogs with light to red wheaten colour (Mann et al. 1966).

There is a vast phenotypic variability between the domestic dog breeds due to selection pressure of the breeders. Thus, genomic regions influencing breed-specific appearance can be detected. At the same time artificial selection resulted in an increased level of inbreeding (Lindblad-Toh et al. 2005), that are commonly estimated via pedigrees. Nevertheless, this estimation is sometimes influenced by the possible incompleteness or inaccuracy of pedigrees. The quantification of the genomic inbreeding coefficient (F) based on SNP or sequence data is widely acknowledged as an alternative, higher precision measure (Peripolli et al. 2016).

Runs of homozygosity (ROH) are continuous segments in the DNA-sequence of diploid organisms. Due to the high correlation with autozygosity (r~0,7) (McQuillan et al. 2008), they can be implemented as a measurement for individual autozygosity. Through mating of related individuals chromosomal segments are passed to the progeny what results in long homozygous regions of the genome that are identical by descent (IBD) (Curik et al. 2014). Long ROH originate from a more recent ancestor, shorter ROH from a more distant one. An increase of ROH derives, alongside with genetic drift and population bottlenecks, from intensive artificial selection and inbreeding. Through ROH detection the genetic relationships and inbreeding levels can be calculated, offering a better understanding of population structure and history (Ferenčaković et al. 2013a), and insights on parts of the genome regulating traits that control breed specific appearance can be provided (Boyko et al. 2010)

The length and number of ROH vary widely between dog breeds, with estimates of mean autozygosity between 7,5% in Jack Russel Terriers to 51% in Boxers (Boyko et al. 2010). Further, the number and length of ROH can be influenced by some parameters and thresholds defined during the analysis (Howrigan et al. 2011). Results from Ferenčaković et al. (2013b) demonstrated changing inbreeding coefficient estimates reliant to the number of heterozygous genotypes permitted. Moreover, the density of the SNP chip and the frequency of genotyping errors can impact the number of ROH detected. The aim of this paper was to identify excessively homozygous regions in Rhodesian Ridgeback dogs and to examine their potential influence on the population.

Material and methods

DNA from 24 Rhodesian Ridgeback dogs from Austria was obtained and genotyped with the whole genome Illumina CanineHD Whole-Genome Genotyping BeadChip containing 173,662 SNPs.

The quality control was done with PLINK 1.9 software (Chang et al. 2015; Purcell et al. 2007) when records with more than 12% missing genotypes and SNPs with more than 10% missingness and outside of the 10E-7 for the Fisher's exact test p-value were deleted. SNPs on sex chromosomes and those with unknown position were also deleted. One of the dogs and 12,215 SNPs were removed after quality control.

The above quality control limits could be considered as standard limits in animal breeding. Two *ad hoc* adjustments were done however. The first one was the slight increase of missingness per sample to 12%, as the standard 10% limit would narrowly exclude two of the remaining 23 dogs. This trade off was to include as many dog in the analysis as possible. The second adjustment was the omission of the minor allele frequency check for the purposes of the genomic inbreeding analyses. Here we looked for long, continuous homozygous segments, so the removal of monomorphic SNPs would likely introduce bias into our evaluation.

The individual genomic inbreeding coefficients (F_{ROH}) were calculated using the runs of homozygosity (ROH) approach, based on the formula proposed by McQuillan et al. (2008)

$$F_{ROH} = L_{ROH}/L_{AUTOSOME},$$

where L_{ROH} is the total length of all ROH in the genome of an individual while $L_{AUTOSOME}$ refers to the specified length of the autosomal genome covered by SNPs on the chip (here 2,201.66 Mb). Five inbreeding coefficients were calculated: F_{ROH}>1Mb, F_{ROH}>2Mb, F_{ROH}>4Mb, F_{ROH}>8Mb, and F_{ROH}>16Mb defined by the minimum ROH lengths being higher than 1, 2, 4, 8, or 16Mb, respectively.

The cgaTOH program (Zhang et al. 2013) was used to identify ROH segments. ROH islands were identified as partial overlaps of ROH segments in more than half of the genotyped individuals.

The quality control parameters for ROHs were considered according to Ferenčaković et al. (2013b), according to the quality control values of the 50k SNP chip.

Results and discussion

Table 1 illustrates the occurrence of ROH islands of different length and its corresponding inbreeding values (F_{ROH}) within the analysed dogs.

The ROH islands harbored a large number of genes. Nevertheless, only some of them could be unambiguously connected to the Ridgebacks, due to lacking information or publications. The ridge associated genes FGF4 (48.41-48.42 Mb) and ORAOV1 (48.48-48.49 Mb) were identified homozygous at chromosome 18. The ridge derived from a duplication of the FGF3, FGF4, FGF19, ORAOV1 gene complex and is closely related to the occurrence of dermoid sinus. The inheritance of the ridge is dominant and completely penetrant according to Salmon Hillbertz et al. (2007). The same study reported that the genetic predisposition to dermoid sinus in Ridgeback dogs is linked to homozygosity at ridge locus. This malformation is characterized by a tubular cavity with internal keratin and hair, as well as abnormal hair follicles with several hair shafts and sebaceous glands. Accordingly, 13 of 15 homozygous dogs suffered from dermoid sinus, but were also heterozygous in one particular SNP within this complex. Dogs suffering from dermoid sinus are commonly excluded from breeding (Salmon Hillbertz et al. 2007). Further, the gene associated with dog body size, HMGA2

Table 1. Overview of the genomic inbreeding and occurrence of ROH islands

	F_{ROH}[1]	# of ROH segments[1]	Length of segments[1] in Mb	# of ROH islands	Length of ROH islands[1] in Mb
ROH > 1 Mb	0.17 (0.06)	103 (33)	3.6 (3.5)	47	1.6 (1.7)
ROH > 2 Mb	0.14 (0.06)	59 (16)	5.5 (3.9)	20	2.0 (2.0)
ROH > 4 Mb	0.11 (0.05)	32 (7)	8.3 (4.7)	6	4.14 (3.0)
ROH > 8 Mb	0.07 (0.04)	13 (4)	13.0 (5.1)	1	8.6 (na[2])
ROH > 16 Mb	0.03 (0.02)	3 (2)	22.0 (6.8)	0	na[2] (na[2])

[1] – means and standard deviations; [2] – data missing or could not be computed due to a single observation

(8.35-8.49 Mb) (Boyko et al. 2010), was found on chromosome 10 in the analyzed population of Ridgeback dogs. Additionally, the allele for floppy ears is located near MSRB3 (7.89-8.04 Mb) on chromosome 10. Wild canines are invariably characterized by erect ears, whereas the domestic dog breeds present various ear shapes. Floppy-ears are derived from a rapid decrease in heterozygosity at this region (Boyko et al. 2010). This can be validated by our identification of a ROH island in this area. The myostatin gene MSTN (0.73 Mb) coding for muscle patterns was detected on chromosome 37. Double muscling is associated with a mutation of this gene, causing a new "bully" phenotype in the Whippet (Wayne et al. 2007). Cyclooxygenase-2 (COX-2), an indicator gene for kidney-health-status in young dogs (Yabuki et al. 2016) was identified on chromosome 7 at 19.67 Mb. Studies on knockout and knockdown mice proved the gene to be a causative part of normal kidney development (Norwood et al. 2000, Seta et al. 2009) due to its regulating function of renal blood flow and glomerular filtration rate in the macula densa of the thick ascending limbs in the kidney (Hao and Breyer 2008). Low or undetectable renal COX-2 expression can be found in young, chronic kidney diseased dogs (Yabuki et al. 2016). PDE6 G, part of the PDEcomplex (PDE6A, PDE6B, PDE6G and PDE6D), related to retinal atrophy in some breeds was identified at chromosome 9 located at 0.53 Mb. The initial stage of this disease is named rod-cone dysplasia and can be observed in two types, rcd1 and rcd2. The latter is associated with an insufficient cyclic guanosine monophosphate-phosphodiesterase (cGMP-PDE) activity and in case of rcd1, with a nonsense mutation in PDE6B (Wang et al. 1999). PDE6 G, however, was thought to be linked to rcd2 (Wang et al. 1999), which is characterized by an increased cGMP and an inaccurate cGMP-PDE activity. (Woodford et al. 1982). Yet, the gene was excluded as a potential rcd2 elicitor (Wang et al. 1999). Further, APOH on chromosome 9 (14.08 Mb) was tightly linked to the progressive rode cone degeneration (prcd) locus and under suspicion to be a causative part of the autosomal recessive disease. This connection was disproved by a linkage analysis however (Gu et al. 2017). The NOS2 gene located in a ROH island on chromosome 9 (42.17 Mb) and the, interferon gene IFNG on chromosome 10 (10.41 Mb) were identified as parts of the immune response in canines. Canines play a role as host for these protozoa, further the disease can be transmitted via sandflies and infect humans (Nascimento et al. 2015). Based on the relevance of this disease, a study on dogs was performed by Nascimento et al. (2015) reporting that symptomatic dogs show lower IFNG and iNOS expression compared to asympomatic dogs revealing that an activated expression of these genes (IFNG, iNOS and TNF-α) results in elimination of the parasite. On chromosome 38 a part of the signaling lymphocyte activation molecule (SLAM) family SLAMF1 (21.65 Mb) was identified. SLAM functions as a cellular receptor for morbilviruses that are provoking human and animal diseases resulting in severe immunosuppression and lymphopenia (Tatsuo et al. 2001). Distemper infected canines exhibit distressed gastrointestinal, respiratory and urinary tracts; skin; and the central nervous system (CNS) (Wenzlow et al. 2007).

Within the detected genes, only the latter have a proven function in dogs yet. However, other genes with known functions in other species were identified in the ROH islands described below. The dog has more disease syndromes in common with humans than any other domestic or laboratory species (Ostrander and Wayne 2005). Therefore, one can hypothesize gene function relatedness between dogs and humans regarding diseases.

On chromosome 9, the gene GCGR (0.45-046 Mb) coding for a glucagon receptor was identified. Regarding the high occurrence of diabetes, counteracting the glucagon receptor is mediated to lower hyperglycaemia (Yang et al. 2007). CRYBA1, a gene expressed in the eye, was found on chromosome 9 (43.37 Mb) serving as structural protein for the lenses, keeping them transparent. Mutations of CRYBA1 are proofed to be involved in ocular development disorders (Graw 2003). On chromosome 10 the TIMELESS gene (0.67 Mb), regulating the manifestation of the circadian rhythm (~24 h) in D*rosophila,* was detected (Bae et al. 1998). A mutation of this gene generates 33 h rhythms in this species (Rothenfluh et al. 2000). On chromosome 12 OSTM1 (65.09-65.12 Mb), also called grey lethal gene, was detected. A 2 bp deletion in exon 3 of this gene is involved in autosomal recessive malignant infantile osteopetrosis (ARO) in humansThe protein scribble is encoded by the gene SCRIB detected on chromosome 13 (37.36 Mb). According to Norman et al. (2011) the protein acts as a tumour suppressor in *Drosophila* and mammals.

Conclusion

Runs of homozygosity analyses were performed on a small population of Rhodesian Ridgeback dogs. Overlaps in ROH segments in more than half of the genotypes were identified as ROH islands, which could be also interpreted as signatures of selection. Upon closer examination the gene determining the breed

characteristic ridge phenotype was found in one of the islands. Other ROH island harboured the genes related to the floppy ear type, body size, muscling patterns and health.

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